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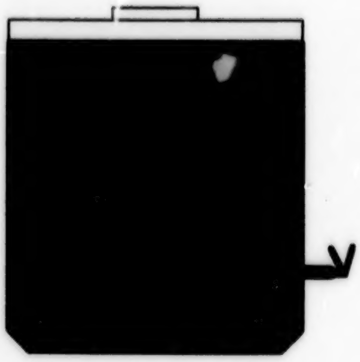
Evaluation of Batch Marking Techniques for Culturing Endangered Razorback Sucker and Colorado Squawfish



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88% vs. 60% after 5 months, and 74% vs. 60% after 15 months). This was due to lighter pigmentation of the Colorado squawfish. Haines and Modde (1996) marked 35-45 mm TL Colorado squawfish on the dorsal surface with red elastomer. They reported 98% retention after 21 d and 85% after 142 d.

Although not always significant, the red and orange marks were more consistently recognized than blue and green. Most often misidentification was the result of not seeing any mark rather than confusing one mark for another. Occasionally, however, blue was

Evaluation of Batch Marking Techniques for Culturing the Endangered Razorback Sucker and Colorado Squawfish

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below the dorsal fin, and on the caudal peduncle. Juvenile bluegill lost elastomer marks applied to the dorsal surface of the skull but retained all other marks. Bonneau et al. (1995) reported elastomer retention of nearly 100% after 4 months for implants in juvenile and adult salmonids on the top of the head, adipose tissue behind the eye, adipose fin, dorsal fin, pectoral fin, and caudal fin.

During the course of our study, razorback sucker mortality averaged 12%. The mortality was spread among fish of all groups and apparently unrelated to marking (Table 3). Colorado squawfish

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Disclaimer

The opinions and recommendations expressed in this report are those of the authors and do not necessarily reflect the views of the U.S. Fish and Wildlife Service, National Park Service or the Recovery Implementation Program.

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Recommendation

The greater retention time and efficiency in marking makes the use of freeze branding the practical replacement for PIT tagging during the first two growing seasons of hatchery reared fishes.

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Executive Summary

Young razorback sucker *Xyrauchen texans* and Colorado squawfish *Ptychocheilus lucius* were marked by freeze branding and fluorescent elastic polymer (elastomer). The elastomer implant was located at the base of the anal fin and on the operculum (razorback sucker) or lower jaw (Colorado squawfish) and consisted of one of four colors: green, blue, red, or orange. Freeze branding had retention rates of 99% after 15 months for both species. Elastomer recognition was better for Colorado squawfish (grand mean 74%) than razorback sucker (grand mean 60%) after 15 months. No elastomer location proved to be superior to the others. Red and orange had the best recognition, blue the poorest. The red and orange elastomer implant for young Colorado squawfish had five month retention rates of 95%. Given these results the following recommendations are proposed.

1. Freeze branding had excellent retention for 15 months for both razorback sucker and Colorado squawfish and could easily substitute for the use of internally implanted passive integrated transponder (PIT) tags following the first growing season.

2. Elastomer recognition, although acceptable for short time periods (red and orange) on Colorado squawfish, are not recommended for marking fishes if identification is desired beyone 12 months.

3. One person implanting elastomer at two locations on each fish and one person anesthetizing and handling fish can mark 130-140 fish per h. Approximately three times that number can be freeze-branded per h. The greater retention time and efficiency in marking makes the use of freeze branding the practical replacement for PIT tagging during the first two growing seasons of hatchery reared fishes.

Introduction

Artificial propagation is an important tool for recovering endangered razorback sucker *Xyrauchen texans* and Colorado squawfish *Ptychocheilus lucius* within their native range in the Colorado River Basin (Rinne et al. 1986; Johnson and Jensen 1991; Modde et al. 1995). Small numbers of founders in the hatchery stock make the genetic contribution of each individual important to maintaining genetic diversity. The identification of offspring from each paired mating is necessary for the maintenance of pedigree records for brood stock development and equalization of family lots for achieving maximum effective population size for reintroduction or augmentation of wild populations (Echelle 1991). Unique marking of family lots contributes to the efficiency of the fish culture facility by allowing the mixing of lots in large growout ponds. Internally implanted passive integrated transponder (PIT) tags (Prentice et al. 1990) are ideal for marking large fish, but the tags are expensive (approx. $4.00 per tag), the fish must be at least 100 mm total length (TL), and an electronic scanner is required to read the tag. The use of PIT tags to mark juvenile fish can add significant costs to propagation, especially when large numbers of age-0 and age-1 fish may be excess to the genetic refugia target numbers. A simple and inexpensive batch mark that can be recognized after one or two years would substantially reduce cost and increase pond use efficiency.

Potential marks include freeze branding and elastomer injection. Freeze branding is a widely used technique for marking small salmonids (Laird et al. 1975; Mighell 1969; Fay and Pardue 1985; Knight 1990), channel catfish *Ictalurus punctatus* (Brock and Farrell 1977), and walleye *Stizostedion vitreum* (LaJeone and Bergerhouse 1991), but it has not been tested for razorback sucker and Colorado squawfish. A fluorescent elastic polymer (elastomer) was recently developed by

NorthWest Marine Technology, Incorporated[1]. Bonneau et al. (1995) used this technique to mark salmonids, Dewey and Zigler (1996) to mark bluegill *Lepomis macrochirus*, and we used it to mark small (30-70 mm TL) Colorado squawfish (Haines and Modde 1996). A successful batch mark must have the capability of providing several different marks and last for one or two growing seasons.

This study compared the effectiveness of freeze branding and elastomer injection for use in short-term identification of family lots of juvenile razorback sucker and Colorado squawfish. Specifically, we addressed three objectives: 1) to compare freeze branding and visible implant fluorescent elastomer tags for rate of retention during a 15 month period, 2) to determine if the four available colors of elastomer are equally detectable, and 3) to compare the operculum (razorback sucker) or lower jaw (Colorado squawfish) and base of the anal fin as locations for an elastomer implant.

Methods

The freeze branding apparatus used in this study was similar to one described by LaJeone and Bergerhouse (1991). It contained a 2 L reservoir of liquid nitrogen and a metal branding iron extending through the wall of the reservoir. The branding iron terminated in a V-shape. The left side of the fish, anterior to the origin of the dorsal fin, was held against the iron for approximately 6 s for marking. Four colors (green, blue, red, and orange) of fluorescent elastomer, obtained from Northwest Marine Technology, Incorporated, were injected subcutaneously with a 1.0 ml insulin syringe and 28-gauge needle to one of two locations on the fish.

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[1] Mention of trade names or commercial products does not constitute endorsement by the Recovery Implementation Program for Endangered Fish Species in the Upper Colorado River Basin.

Two-hundred forty razorback sucker (mean TL 127 mm) and 1,015 Colorado squawfish (mean TL 150 mm), all containing PIT tags for individual identification, were divided into three groups per species. Each group (hereafter called trial) was assigned a marking date between 6 and 13 June 1995 (Table 1). Each fish had three potential marking locations: the left side for freeze-branding, the left operculum (razorback sucker) or lower jaw (Colorado squawfish), and the base of the anal fin for elastomer implant. The treatments were assigned at random to each fish. One-half of each lot received a freeze brand and one-half no brand (control). One-fifth of each lot received one of the four colors or no mark (control) on the operculum or lower jaw and base of the anal fin. For all trials, the same person marked with freeze-brand (Steven Severson) and elastomer (Ronald Nichols) to reduce variation associated with inconsistencies in application. Prior to marking, the fish were anesthetized with tricain methanesulfonate (MS-222). After marking, razorback sucker were placed in a 0.08 hectare pond and Colorado squawfish in two 0.08 hectare ponds. The fish were harvested, PIT tags read, and marks identified after five and 15 (Colorado squawfish) or 16 (razorback sucker) months. The elastomer marks were observed under an ultraviolet lamp in a darkened room.

The elastomer data were analyzed using two-way analysis of variance (2 marking locations X 4 marks) and blocked by trial. There was no replication within blocks; thus, we were unable to test for interactions between marking locations and marks. Mark retention rates were log transformed. The software SYSTAT (Wilkinson 1990) was used to compute the statistics.

Table 1. - Dates of marking fish, numbers of fish marked, and dates of checking fish for recognizable marks. rbs = razorback sucker, csf = Colorado squawfish.

Trial	Species	Date marked	Number marked	1st Date checked	2nd Date checked
1	rbs	6 Jun 95	80	8, 13 Nov 95	23 Oct 96
1	csf	6 Jun 95	343	27 Nov 95	24 Sep 96
2	rbs	7 Jun 95	80	8, 13 Nov 95	23 Oct 96
2	csf	8 Jun 95	335	27 Nov 95	24 Sep 96
3	rbs	8 Jun 95	80	8, 13 Nov 95	23 Oct 96
3	csf	13 Jun 95	335	27 Nov 95	24 Sep 96

Results and Discussion

The results of the experiment are summarized for razorback sucker in Tables 2 and 3 and Colorado squawfish in Tables 4 and 5. The V-shaped freeze brand was recognizable on greater than 99% of treated fish after 15 months for both the razorback sucker and Colorado squawfish. The freeze brand blackened tissues and deformed scales. On previous occasions we used an O-shaped brand and found it just as recognizable as the V, but a horizontal bar-shaped brand was not recognizable. The edges of the bar became distorted as the fish grew and blended with the fish's natural markings. Freeze brand retention rates were similar to those reported by others. LaJeone and Bergerhouse (1991) reported 95% of branded walleye retained recognizable marks after 5 months in a rearing pond. Fay and Pardue (1985) reported 92% retention of a T-shaped brand on rainbow trout *Oncorhynchus mykiss* after 22 weeks. They also noted that 60% of the brands had deteriorated to poor quality.

Recognition of the elastomer had mixed results, depending on the species, color, and location. Recognition was easier for Colorado squawfish than razorback sucker (grand means of

Table 2. - Percent recognition five months after marking young razorback sucker with elastomer implanted at the base of the anal fin and on the operculum and freeze branding. Note: percentages consist of means of three trials.

Location	Color	Number observed (%)	Corr. ident. (%)	Incorr. ident. (%)	Mortalities
anal fin	non	48	100	0	0
	grn	45	53	47	6
	blu	47	68	32	2
	red	46	85	15	4
	orn	47	94	6	2
	mean[a]		75	25	
opercle	non	48	100	0	0
	grn	46	39	61	4
	blu	46	30	70	4
	red	48	46	54	0
	orn	46	63	37	4
	mean[a]		45	55	
side	non	118	99	1	1
	frz	115	99	1	4

[a] mean of grn, blu, red, orn

ANOVA showed that anal fin had significantly better retention rate than the opercle (P = .004), but no significant difference was found among colors of elastomer (P = .159).

Table 3. - Percent recognition 16 months after marking young razorback sucker with elastomer implanted at the base of the anal fin and on the operculum and freeze branding. Note: percentages consist of means of three trials.

Location	Color	Number observed (%)	Corr. ident. (%)	Incorr. ident. (%)	Mortalities
anal fin	non	44	98	2	8
	grn	39	34	66	19
	blu	43	41	59	10
	red	41	86	14	15
	orn	44	91	9	8
	mean[a]		63	37	
opercle	non	45	95	5	6
	grn	41	60	40	15
	blu	43	36	64	10
	red	40	67	33	17
	orn	42	64	36	13
	mean[a]		57	43	
side	non	106	100	0	12
	frz	105	100	0	13

[a] mean of grn, blu, red, orn

ANOVA showed no significant difference between anal fin and opercle implant (P = 0.91), or among colors (P = 0.07).

Table 4. - Percent recognition five months after marking young Colorado squawfish with elastomer implanted at the base of the anal fin, on the operculum, and freeze branding. Note: percentages consist of means of three trials.

Location	Color	Number observed	Corr. ident. (%)	Incorr. ident. (%)	Mortalities (%)
anal fin	non	201	99	1	3
	grn	195	81	19	3
	blu	203	67	33	0
	red	198	98	2	1
	orn	196	97	3	3
	mean[a]		86	14	
jaw	non	204	100	0	2
	grn	195	82	18	3
	blu	197	87	13	4
	red	201	94	6	0
	orn	196	96	4	1
	mean[a]		90	10	
side	non	503	99	1	2
	frz	490	99	1	2

[a] mean of grn, blu, red, orn

ANOVA showed no significant difference between anal fin implant and jaw implant (P = .330), but showed significant among colors (P = .018); Tukey honestly significant difference multiple comparison test showed orange had significantly better recognition than blue (P = .032).

Table 5. - Percent recognition 15 months after marking young Colorado squawfish with elastomer implanted at the base of the anal fin, on the operculum, and freeze branding. Note: percentages consist of means of three trials.

Location	Color	Number observed	Corr. ident. (%)	Incorr. ident. (%)	Moralities.[b] (%)
anal fin	non	39	100	0	81
	grn	49	74	26	76
	blu	36	49	51	82
	red	49	95	5	75
	orn	32	77	23	84
	mean[a]		74	26	
jaw	non	37	100	0	82
	grn	40	62	38	80
	blu	44	45	55	79
	red	43	88	12	79
	orn	41	96	4	79
	mean[a]		73	27	
side	non	100	99	1	81
	frz	105	99	1	79

[a] mean of grn, blu, red, orn

[b] Colorado squawfish suffered heavy mortality (79%) between 11 July and 16 September 1996, the result of bacterial septicemia unrelated to marking.

ANOVA showed no significant difference between anal fin and jaw implants (P = 0.82), but showed significant difference among colors (P = 0.003). Tukey honestly difference multiple comparison test showed red (P = 0.001) and orange (P = 0.002) had significantly better recognition than blue.

88% vs. 60% after 5 months, and 74% vs. 60% after 15 months). This was due to lighter pigmentation of the Colorado squawfish. Haines and Modde (1996) marked 35-45 mm TL Colorado squawfish on the dorsal surface with red elastomer. They reported 98% retention after 21 d and 85% after 142 d.

Although not always significant, the red and orange marks were more consistently recognized than blue and green. Most often misidentification was the result of not seeing any mark rather than confusing one mark for another. Occasionally, however, blue was confused with green and red with orange. When viewing the elastomer, the ultraviolet lamp projected a bluish color and may have accounted for the low detection rate of the blue elastomer. No single elastomer implant location was best. For Colorado squawfish, the jaw and the base of the anal fin appeared equally suitable. For the razorback sucker, the anal fin site was best after 5 months, but after 15 months both sites were approximately equal.

Tissue growth over the elastomer during the course of the study accounted for some loss of mark visibility at the anal fin site. The razorback sucker grew from 21.5 to 247.0 g over 15 months and the Colorado squawfish from 23.5 to 141.0 g over 16 months. On 29 November 1995 we dissected seven Colorado squawfish that had originally been marked with elastomer, and in several instances exposed more elastomer than could be seen when viewed under ultarviolet lamp. At the lower jaw site we were unable to expose any more elastomer than we originally observed. In a number of instances elastomer implanted at the base of the anal fin migrated to tissues between anal fin rays after five months. These marks were easily visible 15 months after marking.

Dewey and Zigler (1996) found elastomer retention for adult bluegill was more than 99% after 6 months for fish marked on the dorsal surface of the skull, at the insertion of the dorsal fin, below the dorsal fin, and on the caudal peduncle. Juvenile bluegill lost elastomer marks applied to the dorsal surface of the skull but retained all other marks. Bonneau et al. (1995) reported elastomer retention of nearly 100% after 4 months for implants in juvenile and adult salmonids on the top of the head, adipose tissue behind the eye, adipose fin, dorsal fin, pectoral fin, and caudal fin.

During the course of our study, razorback sucker mortality averaged 12%. The mortality was spread among fish of all groups and apparently unrelated to marking (Table 3). Colorado squawfish suffered heavy mortality (79%) between 11 July and 16 September, the result of bacterial septicemia. The mortality was also spread among fish of all groups and apparently unrelated to marking (Table 5).

Our experience was that between 130 and 140 fish per h could be marked by one person implanting elastomer at two locations on each fish and one person anesthetizing and handling fish. Approximately three times that number can be freeze-branded per h by a single individual.

Conclusions

1. Freeze branding had excellent retention for 15 months for both razorback sucker and Colorado squawfish.
2. Elastomer recognition, although acceptable for short time periods (red and orange) on Colorado squawfish, were not suitable for marking fishes if identification is desired beyond 12 months.
3. One person implanting elastomer at two locations on each fish and one person anesthetizing and handling fish can mark 130-140 fish per h. Approximately three times that number can be freeze-branded per h.

Recommendation

The greater retention time and efficiency in marking makes the use of freeze branding the practical replacement for PIT tagging during the first two growing seasons of hatchery reared fishes.

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